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COCOON BUILDING AND THE PRODUCTION OF SILK BY THE MATURE LARVA OF *DIANOUS COERULESCENS* GYLLENHAL (COLEOPTERA: STAPHYLINIDAE)

By M. F. Jenkins

(Department of Zoology, University of Bristol)

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With 8 Text-figures

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I. Cocoon Building and Pupation

The last instar larva of *Dianous coerulescens* Gyllenhal spins a cocoon within which it pupates. In nature the larva spins the cocoon beneath stones which lie propped against the bank of the stream or in niches of large mossy stones in mid-stream. Cocoons may be found in May and throughout the summer months but not later than the middle of October. The cocoon is white in colour and is a double structure. The inner envelope is regularly elliptical and measures 8 by 5 mm.; the outer envelope is of irregular shape, often measuring 18 by 12 mm. The outline of the pupa is just visible through the double envelope.

Cocoon building was observed in the laboratory. The last instar larvae generally spin four or five days after the preceding moult, and at this time they are easily recognised by their greatly distended abdomens. Such larvae were placed singly in simple observation cells floored with damp leaves. Spinning larvae repair a damaged cocoon by daylight but the spinning of a complete cocoon always starts at night. Accordingly observations were made using red light and provision was made to avoid any heating effects from the lamp.

Before spinning, larvae explored all parts of the cell. Sites where leaf fragments projected upwards a little way from the cell wall were always pre-

ferred to those where the larva would have to spin between the upright cell wall and a flat floor of damp leaves. Larvae reject sites which do not allow them to move through their own length.

Silk is produced by paired glands which open on the ninth tergite a little anterior to the bases of the anal cerci. Before spinning, slow peristaltic waves move back along the abdomen and this is followed by the appearance of clear silk globules at the openings of the silk glands. The larva quickly thrusts the dorsal end of the abdomen against part of the substrate so that the anal cerci lie flush with the surface. It then walks away pulling down the abdomen. By this manoeuvre, silk is smeared the length of the anal cerci and is pulled out as two long threads from their tips as the larva proceeds. After walking its own length, the larva stops, lifts the end of the abdomen off the ground, jerks it up and down in a characteristic fashion, and bends the anal cerci against another part of the substrate. The larva again moves off, turning to cover the opposite wall of the site, and by repetition of this behaviour a silken mat is spun which covers and joins together the leaf fragments. While spinning the framework of the outer envelope, the larva walks sideways and backwards, but more generally forwards, continually circling the chosen site. The cerci are mobile and can be depressed to play beneath the abdomen. The larva never supports itself on the framework that it is making but spins standing on the substrate. Gaps in the framework seem to be checked by use of the antennae and mouth parts. The outer envelope is usually completed about five hours after the start of spinning and the larva at once begins to make the inner envelope.

The larva walks little in spinning the inner envelope but continually turns in its own length so that this envelope is relatively near to its body. Such inner envelopes were often completed in four hours, and observation indicates that the cocoon is almost certainly spun within 12 hours in the field. The silk glands are not quite emptied with completion of the cocoon, for larvae can repair tears to the inner envelope, and larvae removed entirely from a completed cocoon can spin a second outer envelope. In such cases, a further inner envelope is not made, and, as larvae may yet remain active for some hours, this seems to be due to exhaustion of the silk supply. Generally, the larva becomes quiescent some hours after completion of the inner envelope, and over a period of 12 hours loses the ability to control movement of the antennae, mandibles and legs. In the laboratory, the whole of the first night after spinning was spent in this so-called " prepupal " phase, and the pupa shed the last larval skin on the second night. The pupal stage is commonly of four days' duration. In the next days pigmentation increases rapidly in the head and abdominal region of the pharate adult, and the insect bursts through the pupal skin and eats its way out of the cocoon some six days after spinning.

II. Anatomy of the Silk Glands

Few beetles are known to spin silken cocoons, but in the Staphylinidae cocoons have previously been noted. Blair (1917) found cocoons of *Stenus similis* Herbst, but does not mention the larval silk glands. Verhoeff (1920) has observed a well developed median gland which opens on to the eighth tergite of beetles of the genus *Leptusa* Kraatz, and this seems to be a silk gland. Mature larvae of *Microglossa* Kraatz also appear to possess a gland opening

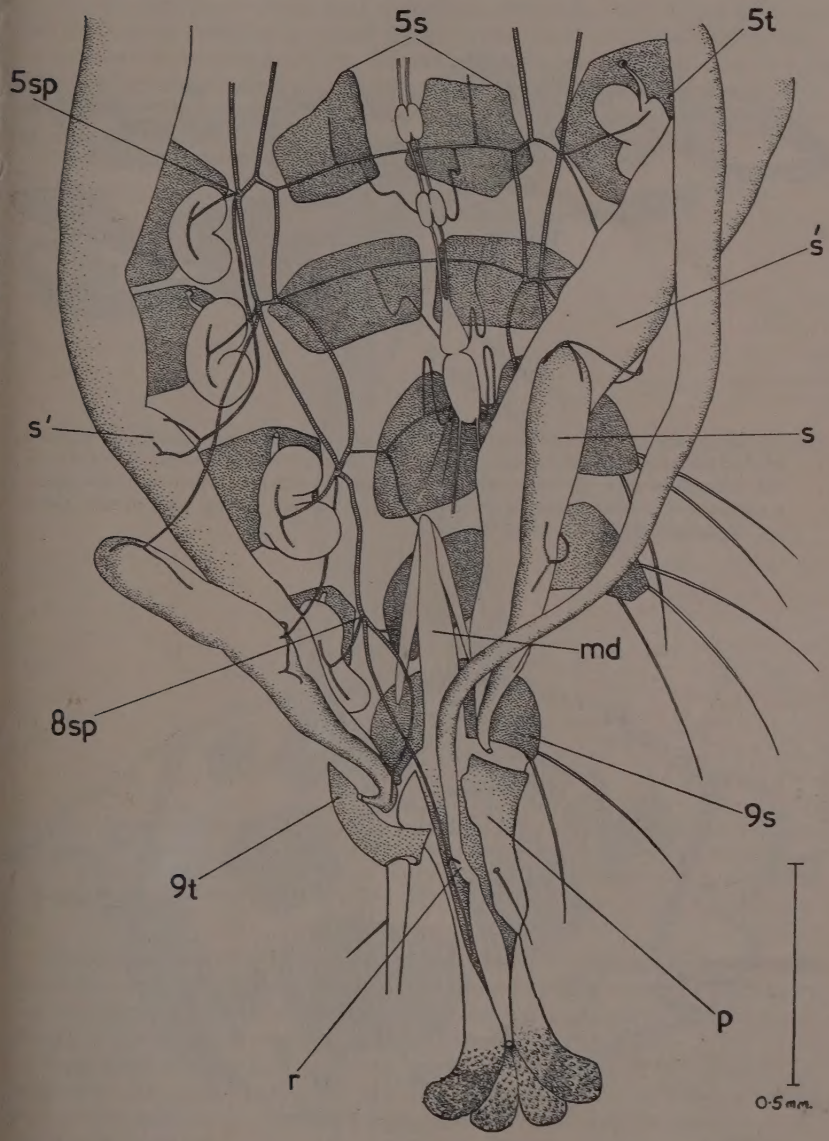

FIG. 1.—Dissection of the posterior region of a mature male larva of *Dianous coerulescens* Gyll. Scale, 0·5 mm. *md*, anlagen of male genital systems (part); *p*, pygopod; *r*, rectum; *s*, smaller dorsal part of the silk gland; *s'*, larger ventral part of the silk gland; 5*s*, 9*s*, sternites; 5*sp*, 8*sp*, spiracles; 5*t*, 9*t*, tergites.

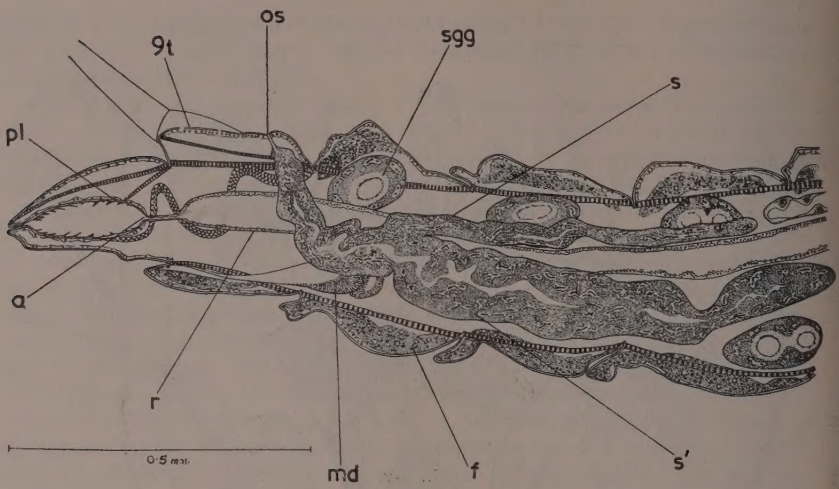

Fig. 2.—Diagrammatic vertical longitudinal section of the posterior abdominal segments of a last instar larva of *Dianous coerulescens* Gyll. Scale, 0·5 mm. *a*, anus ; *f*, fatty tissue ; *md*, anlagen of male genital system ; *pl*, pygopod lobes ; *os*, opening of silk gland ; *r*, rectum ; *s*, smaller dorsal part of silk gland ; *s'*, larger ventral part of silk gland ; *sgg*, segmental gland ; *9t*, ninth tergite.

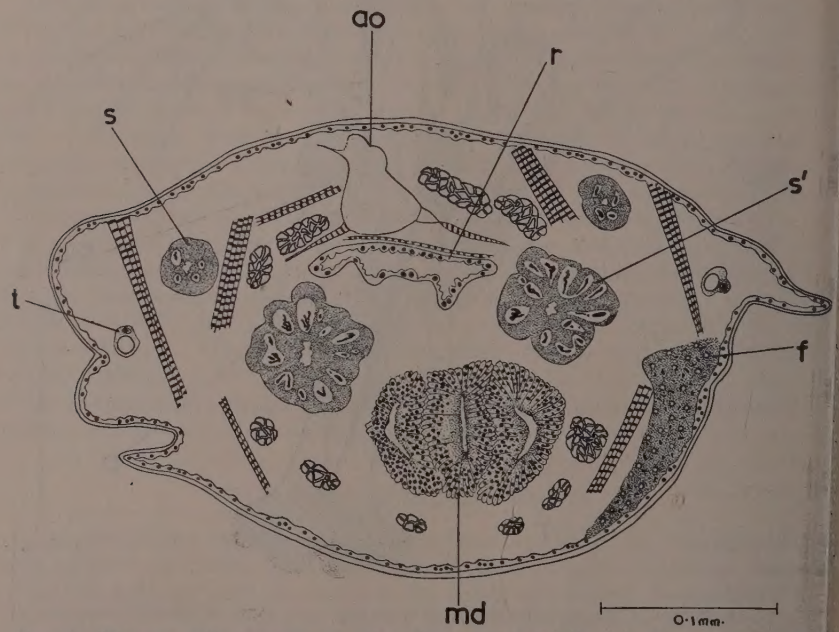

Fig. 3.—Diagrammatic transverse section through the eighth abdominal segment of a mature larva of *Dianous coerulescens* Gyll. Scale, 0·1 mm. *ao*, aorta ; *f*, fatty tissue ; *md*, anlagen of male genital system (part) ; *r*, rectum ; *s*, smaller dorsal part of silk gland ; *s'*, larger ventral part of silk gland ; *t*, trachea.

on to the eighth tergite. Paulian (1941) has observed the spinning of a cocoon by an undetermined larva of the genus *Cardiola* Jacobs.

The present study shows that the silk used to make the cocoons of *Stenus* and *Dianous* is produced in paired glands, which open on to the ninth tergite. In *Dianous* these silk glands are present in the first instar larva, and in both first and last instar larvae the glands extend through five abdominal segments. In the older larva they become greatly swollen and are partly responsible for the characteristically distended abdomen at the end of the final instar.

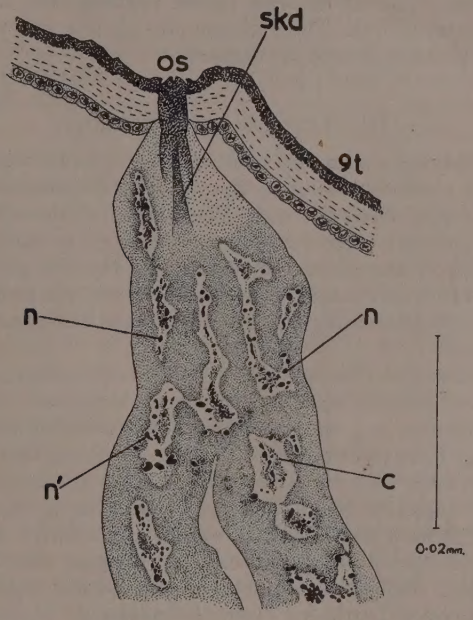

FIG. 4.—Vertical longitudinal section through the opening of a silk gland of a mature larva of *Dianous coerulescens* Gyll., fixed shortly before spinning. Scale 20 μ. *c*, chromatin granules; *n*, spherical nucleolus; *n'*, irregular nucleolus; *os*, external opening of gland on ninth tergite (*9t*); *skd*, silk gland duct.

Throughout larval life the glands are white in colour and lie lateral and somewhat ventral to the rectum. Each gland is subdivided so that its main body lies ventrally and a slender finger-like part lies more dorsally. Both parts of the gland join just before emptying into the common duct (figs. 1, 2, 3).

Both parts of the gland possess a lumen and are cytologically similar. In sections of first instar larvae the spinning glands appear as a syncytium with rounded or oval nuclei embedded in a granular homogeneous cytoplasm. In pre-spinning last instar larvae the nuclei have become enlarged and amoeboid.

There is no membrane about the spinning glands sheathing them and retaining them in position, nor do the glands have any muscular attachments. The glands appear to be held in place by their tracheal supply, which is derived

from the third to eighth abdominal spiracles. The tracheae ramify over the surface of the glands but do not appear to penetrate into them.

There is no structure corresponding to a silk regulator or press, nor have any canal or regulative cell nuclei similar to those described for Verson's glands been seen (fig. 4). Silk may be extruded from the glands as a result of fluid pressure brought about by the peristaltic movements of the abdominal segments which are noticeable in actively spinning larvae.

The silk gland openings are twelfth of a series of paired openings which are found towards the outer anterior borders of the three thoracic and first nine abdominal tergites. The 11 anterior paired openings are those of the well developed segmental glands. These glands also show a syncytial structure in section and may perhaps be used for defence.

III. Material and Methods

Ideally, in studying a secretory mechanism one would wish to observe the development of a particular secretory droplet from its moment of origin to the point of its extrusion from the cell. The nature of the spinning glands of *Dianous* precludes such observation. Accordingly, in addition to making preparations to show the general appearance of the silk glands through the secretory cycle, various techniques were used to reveal the position and number of the different cytoplasmically formed bodies, and any changes exhibited by them.

Last instar larvae of *Dianous* were collected from mossy stones in a fast-flowing stream at Failand, Somerset. Spinning larvae are most easily found in the summer months, and cannot be found after mid-October.

The following fixatives were used: Bouin, Dubosq-Brasil, Susa, Zenker, and Flemming's fluid without acetic acid. Zenker fixation gave quite good results but the Feulgen technique worked better after alcohol-sublimate fixation. The best fixation was obtained with Bouin's fluid; this was probably due to the good penetration of picric acid. All staining was with Heidenhain's iron haematoxylin. Cellosolve or methyl-benzoate was used in place of the higher alcohols to avoid hardening during dehydration. Material was sectioned after embedding in a paraffin wax containing 5 per cent. diethylene-glycol-distearate. Sections were cut at thicknesses of 4 to 8 μ with an ordinary rocking microtome.

Two methods were tried in order to demonstrate mitochondria. Firstly, Benoit's fixative (potassium dichromate 5 per cent., 6 parts; osmium tetroxide 2 per cent., 5 parts; mercuric sublimate, 5 per cent. made up in physiological saline, 5 parts; uranium nitrate 4 per cent., 4 parts) was employed followed by haematoxylin staining, but with mediocre results. Secondly, Benda's process as modified by Grassé was successfully used. Lesperon (1937) regards this technique as specific for revealing mitochondria in silk glands.

Material was fixed for a week in Flemming's fluid without acetic acid. After an hour's washing with tap-water it was placed in a mixture of equal parts of 1 per cent. acetic and 1 per cent. chromic acids. A further 24 hours in a 2 per cent. potassium dichromate solution was followed by a 12-hour wash in tap-water and a rapid alcohol dehydration. After a short embedding, sections were cut at 5 μ. The wax was removed and sections placed in 4 per cent. iron alum

for 24 hours. This was followed by 24 hours' immersion in a freshly prepared 12 per cent. aqueous solution of sodium alizarin-sulphonate. After a rinse in cold distilled water, sections were put into a crystal violet solution (2 per cent. solution in 95 per cent. alcohol with an equal amount of aniline water added at the moment of use) at 50° C. for not more than 2½ minutes. A few minutes differentiation in 30 per cent. acetic acid yielded sections of a clear blue-violet colour. A wash in tap-water brings up the colour of the alizarine, and mitochondria show as intensely violet bodies against a red-brown background. The preparation is dehydrated as quickly as possible, and after a minimal time in the alcohol the sections were passed through xylol and mounted in Canada balsam.

The Golgi bodies were revealed by using Hirschler's modification of Kolatchev's technique. Glands were fixed for 24–48 hours in a mixture of 2 per cent. osmium tetroxide 8 c.c.; 1 per cent. chromic acid 4 c.c.; and 3 per cent potassium bichromate 4 c.c. After a short wash in tap-water, the material was placed in 2 per cent. osmium tetroxide at 38°–40° C. for 4–5 days. Golgi material stained an intense black. The Feulgen method was also used. Sections of material fixed in a sublimate-alcohol fluid (equal parts of saturated mercuric chloride solution and absolute alcohol) were passed through graded alcohols to water and then placed in normal hydrochloric acid for one minute. Sections were then put into normal acid at 60° C. for 4–15 minutes. After a further minute in the cold acid and a rinse in distilled water, sections were put in Schiff's reagent. After oue and a half hours in the reagent, sections were washed three times in sulphurous water. They were shaken in each bath for two minutes. This restored the colour of the fuchsin, and after a five minute wash in tap-water sections were dehydrated and mounted in balsam.

Finally, sections of material fixed with Helley were stained with Unna's carbol-pyronin-methyl-green mixture. Brachet (1940) has shown that ribonucleic acid, the cytoplasmic nucleic acid and the principal constituent of nucleoli, gives an intense red-brown colour with the pyronin of this mixture. But, as a ribonuclease enzyme control was not also used, the results obtained by this method can only be considered together with those produced by haemotoxylin staining.

IV. Cytology of the Silk Glands

(1) *The Resting Gland*

The cytology of the resting gland was studied using first and second instar larvae. At this stage the cytoplasm is uniformly homogeneous, finely granular, quite free from vacuoles, globules, or secretory inclusions, and shows no streaming movements. There is a limiting membrane present on inner and outer surfaces of the gland, the nuclei are not greatly branched, and the nuclear membranes, though fine, are distinct.

Sections of the nuclei vary in size, but characteristically show a number of rounded or irregular bodies which stain intensely with haematoxylin, and aggregations of fine granules which, perhaps because of their relative dispersion, appear as a more lightly stained area. With the Feulgen test, all nuclear contents are red. With Unna's stain the larger bodies and all other nuclear inclusions are green, and the cytoplasm is pink. Such a result, however, is no

indication that the larger bodies invariably stain differently from the cytoplasm. It is possible that they are less basophil than the smaller granules, and that they might give a heavier pyronin coloration than the cytoplasm with a suitably used stain. Perhaps the treatment sufficient to wash out the methyl green and substitute pyronin in the cytoplasm is not sufficient to do the same to the larger nuclear bodies. This possibility has not been tested, but it is suggested because a study of haematoxylin- and other Unna-stained sections shows that these larger nuclear bodies migrate into the cytoplasm in the production of silk, and that, at this time, the cytoplasm stains very heavily with pyronin. There are many careful accounts of nucleoli migrating from the nuclei to the cytoplasm of metabolically active but non-mitotic cells, and of their variation in staining properties during this process.

The recent work of Bernhard, Bauer, Gropp, Haguenau and Oberling (1955) may provide an explanation for this variation. These authors state that although nucleoli are generally Feulgen-negative, and no longer stain with pyronin after the action of a ribonuclease, they are frequently surrounded by a more or less dense ring of Feulgen-positive material which is not digested by the enzyme. They made an electron microscope study of the nucleoli of healthy and cancerous mammalian cells. They confirmed the presence of a reticular or filamentous portion ("nucleonema") and more diffuse intra-, and peri-nuclear zones ("pars amorpha" of Clemente Estable). The nucleoli appeared thus after fixation with osmium tetroxide, but after use of fixatives other than osmium tetroxide, the nucleoli appeared as compact bodies. It should be noted that the reticulate appearance of nucleoli in living cells has been observed by use of phase-contrast microscopy, and it has been suggested that the grains of the reticular zone represent the macromolecules of ribonucleoproteins.

In sections of the silk glands of *Dianous*, the larger bodies within the nucleus appear compact and homogeneous with all fixatives used, but these sections are much thicker than those which are used for electron microscopy. Bernhard, Bauer, Gropp, Haguenau and Oberling (1955) state that in sections of their material fixed with Carnoy and prepared for the ordinary light microscope, the nucleoli, although compact, possess an outer zone which is distinctly separated from the inner part. They suggest that this outer zone may be chromatin material condensed about the nucleolus.

Such condensation may occur generally, and if it does, it would explain the differential staining observed within the migrated nucleoli of a wide range of material by Walker and Tozer (1909), and by others. The methyl green staining reaction of the large bodies within the nucleus of the silk glands of *Dianous* may be a reaction of the outer part of these bodies, and the resemblance between these bodies and previously described nuclear contents of insect spinning glands, suggests that they are nucleoli.

Although the nucleoli vary in size, they are evenly scattered throughout the nucleus. The finer granules which may be scattered, or variously aggregated, may represent the chromatin material. The nucleoplasm is very finely granular. The Golgi bodies are small V-shaped or comma-like structures, scattered throughout the cytoplasm and standing in no definite relation to the nucleus. In sections the Golgi material is rather more concentrated towards the outer edges of the gland. Mitochondria appear as minute dot-like bodies uniformly distributed through the gland cytoplasm.

(2) *Active Glands*

From examination of sections of glands fixed shortly before spinning, it seems that great changes occur in this period. The nucleus appears to have become bigger, to be much more branched and elongated, and to possess more nucleoli. There has been no change in number, appearance or distribution of cytoplasmically formed bodies. Some nucleoli seem to be of two types : those which are spherical or nearly so, and those which are most irregular in shape. The appearance of sections suggests that some nucleoli are actively multiplying at this stage.

Fig. 5.—Appearance of part of a silk gland of a mature larva of *Dianous coerulescens* Gyll., fixed with Susa as the cocoon was being spun. Stained with Heidenhain's iron haematoxylin. Parts of two nuclei can be seen in section, and the gland lumen (*l*) is towards the bottom of the drawing. Scale 15 μ. *n*, spherical nucleolus within the nucleus ; *n'*, irregular nucleolus ; *sg*, intensely staining silk globules.

The nuclear membrane cannot be resolved, and many rounded nucleoli appear to be migrating away from the nuclear space towards the cytoplasm (fig. 7).

Even more striking is the appearance of sections of glands fixed as spinning is taking place (fig. 5). In such sections the nuclei stain more lightly than the cytoplasm. The cytoplasm is full of globules of different sizes which stain intensely with haematoxylin. These can only be silk globules, and the normal appearance of the ground cytoplasm between them makes it unlikely that this

appearance is an artifact. No methyl green and pyronin preparations for this phase have been made, but it may be supposed, on the basis of the earlier appearance, that some of these intensely staining bodies are nucleoli, though most are silk globules. The enormous number of these globules make it quite impossible for them to be derived singly by solution of migrated nucleoli, even allowing for the probable multiplication of nucleoli through the spinning phase. The preparations available do not indicate the relationship between migrated nucleoli and the later appearance of these silk globules.

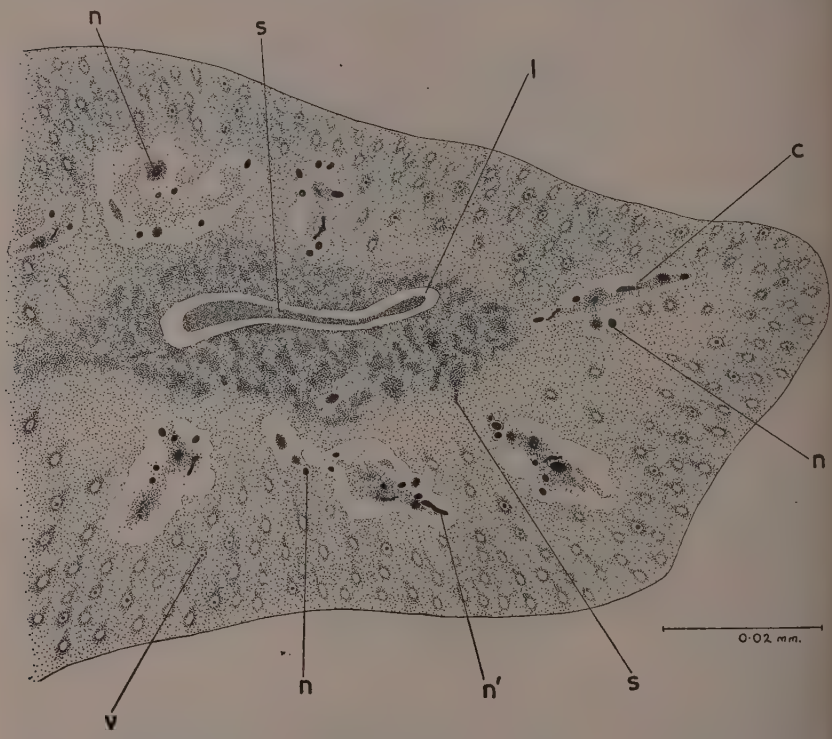

Fig. 6.—Section of innnermost region of a silk gland of *Dianous coerulescens* Gyll., fixed with Susa two hours after the larva had completed the cocoon. Stained with iron haematoxylin. Scale 20 μ. *c*, chromatin ; *l*, gland lumen ; *n*, spherical nucleolus ; *n'*, irregular nucleolus ; *s*, intensely staining, but non globular, formed silk ; *v*, vacuole.

That a relationship exists is, however, quite clear from an examination of sections prepared shortly after the larva has finished spinning the cocoon. The nuclei are no longer greatly branched and they are empty by comparison with their appearance immediately before spinning. The nucleoli have mostly disappeared, but the gland cytoplasm shows strong streaming towards the lumen, and is packed throughout with small vacuoles within some of which small pyronin staining granules may be seen. The sequence obtained from sections made

before, during, and after the spinning of silk, makes it almost certain that the small red staining particles are the remnants of migrated nucleoli. The cytoplasm at this stage stains very heavily with pyronin. The vacuoles in the cytoplasm, by their constant presence after use of various fixatives, do not seem to be artefacts, and it appears that migrated nuceoli, by solution in the cytoplasm, play a part in forming the silk in *Dianous* and in *Stenus*.

What part, if any, is played either by Golgi bodies or by mitochondria is not clear from the material examined. In the sections of larval glands fixed while spinning is taking place, neither may be recognised. But sections of larval glands fixed after spinning do not show either a marked increase, decrease, or redistribution of these bodies as compared with that which has been observed in glands fixed before spinning. The position with regard to the nucleoli is quite

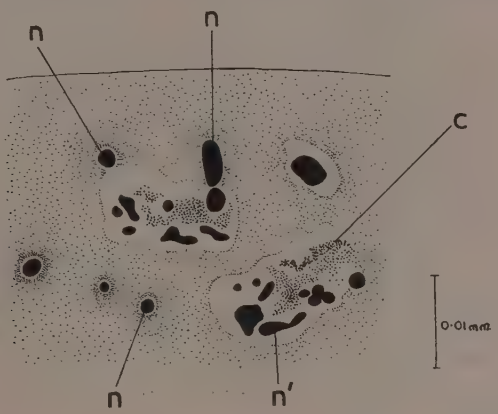

Fig. 7.—Appearance of nuclei in a silk gland of a mature larva of *Dianous coerulescens* Gyll., fixed with Bouin's fluid shortly before the cocoon is spun. Stained wth iron haematoxylin. Scale, 10 μ. *c*, chromatin ; *n*, nucleoli, migrating from a nucleus or in the cytoplasm ; *n′*, irregular nucleolar-like bodies in the nucleus.

different. They are not present in great number in the nuclei after the silk has been evacuated, but are present in great number in the very amoeboid nuclei of glands fixed shortly before spinning, and, at this stage, there are clearly more of them in the nuclei than in the less amoeboid nuclei of glands of younger larvae.

The silk appears to be filtered through the inner membrane of the gland into the lumen. Globules of silk have not been observed crossing the inner membrane of the gland into the lumen, but several preparations show silk globules lying within the gland lumen. At a later stage (fig. 6), the lumen contains a more lightly staining central cord but no separate globules.

V. Discussion

In attempts to arrive at a clear picture of the mechanics of secretion, secretory droplets have been derived from nuclear constituents, from cytoplasmic granules, from mitochondria, and most frequently from the Golgi bodies.

A study of the origin of a particular secretion is difficult primarily because secretory droplets are almost always present in cells in such large numbers that it is impossible to study the development of particular droplets.

The topographical relation of secreted droplets to the Golgi bodies in many tissues is striking and is well known. This, however, is hardly evidence for any necessary connection between Golgi bodies and developing secretory droplets. The polarity of the Golgi material does, in general, coincide with the direction of the secretory current, but cases are known where this polarity is reversed without evidence of a corresponding change in the direction of secretion.

It has recently been shown that Golgi bodies, as usually described, are fixation artefacts. The frequent concentration of secretory material within or about them seems likely to be incidental to their growth during the fixation process, and not to be physiologically significant. Golgi bodies as described from spinning glands of Lepidoptera and Trichoptera are always loop-like or comma-like structures. In Lepidoptera they are found equally in the conducting and reservoir sections of the silk gland. There are no records of a local distribution of Golgi material zoned with forming silk, and in a previous instance where search for such a relationship was made (Beams and Wu, 1929) the result was negative. It is doubtful whether the Golgi apparatus plays any significant part in silk production in *Stenus* and in *Dianous*.

Recently the production of cytoplasmic protein has been much investigated. Siekevitz (1952) has shown that this protein is the product of microsome material. However, the production of microsomes appears to be in some way under nucleolar control, and evidence is accumulating for a scheme of nuclear control of cytoplasmic protein production as suggested by Caspersson (1950). It is now known that development of a nucleolus-heterochromatin complex precedes the onset of increasing concentration of cytoplasmic ribonucleic acid and protein, and the work of Lagerstedt (1949) is a very good demonstration of this relationship.

The nucleolus appears to be a key structure in secretion generally and in silk production in particular. In the account given by Maziarski (1911) for lepidopterous silk glands, an account partly corroborated by later workers, nucleoli were described as being extruded from the nucleus in several ways, according to the intensity of the secretory activity, and dissolving in the cytoplasm. It should, however, be noted that nucleolar extrusion has been denied in the case of the silkworm (Yamanouchi, 1922).

There is no uniformity in the descriptions of how nucleolar material passes through the nuclear membrane. Solvent action at the point of contact with the nuclear membrane has been invoked, and Maziarski believed that nuclear contraction assisted in expulsion of the nucleoli. Maziarski described the escape of nucleoli from the tips of elongated nuclear processes, and this may be observed in *Dianous* also.

It has been suggested that nucleolar extrusions are artefacts peculiar to paraffin sections. In the preparations made during this work, the nuclear membrane is indistinct at the phase of active secretion, but it seems unlikely that nucleoli can have been pushed into the cytoplasm by the impact of the knife, because in serial sections which were carefully orientated they are found emerging in a direction directly opposite to the cutting direction, nor is there any displacement of the irregular nucleoli within the nuclei. Only spherical

nucleoli are found in transit, and they are always surrounded by clear vacuole-like areas.

Although nucleolar extrusion has not been universally described as a normal occurrence in silk gland cells, and although it has been denied in certain instances, nucleolar extrusion in some non-glandular material has never been the subject of debate. Accordingly, it has become possible to argue that, although extrusion may occur in some gland cells, its occurrence may not throw any light upon nucleo-cytoplasmic interchanges or upon secretory activity.

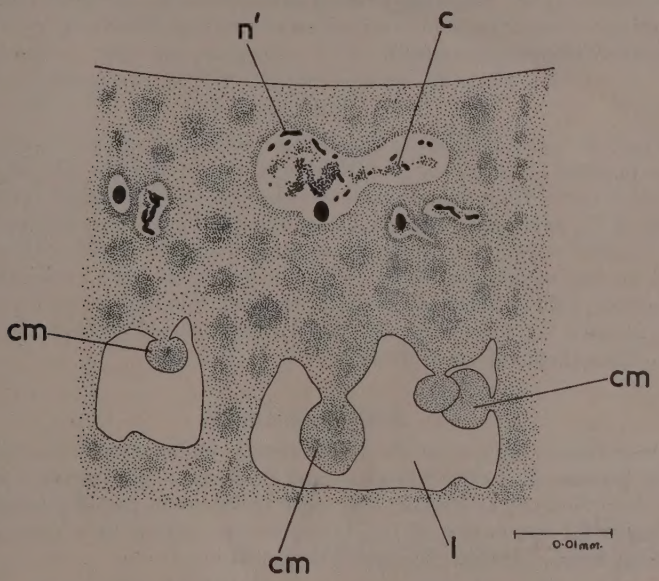

FIG. 8.—Appearance of part of a silk gland of *Dianous coerulescens* Gyll., fixed with Flemming's fluid without acetic acid 12 hours after spinning has been completed. Scale, 10 μ. *c*, chromatin granules; *cm*, cytoplasmic mass, part of the gland substance which is being lost into the lumen, (*l*); lumen already partly occluded; *n'*, irregular nucleolar-like body.

Thus, during mitosis the casting out into the cytoplasm of nucleolar material at the end of prophase is of regular occurrence, although this material does nothing beyond merging with the cytoplasm. It has been argued that if the nucleus can rid itself of nucleolar material at one phase of its cycle it might do the same at other times when for some reason the nucleolar situation needed adjustment, and that the extrusion of nucleoli in gland cells may be unrelated to the origin of secretory material. However, although preparations made during this work seem to show multiplication of nucleoli within nuclei, no figures have been obtained indicating active mitoses by the silk gland nuclei.

In conclusion, it may be noted that the silk glands of *Dianous* and *Stenus* are broken down in the pupal phase and begin to degenerate in the so-called "pre-pupal" stage, the stage which follows the completion of the cocoon by the spinning larva. Sections of the glands prepared in this phase (fig. 8)

show a very different cytology from that which characterises the glands of actively spinning larvae. The nuclei are mostly emptied of the nucleoli, streaming movements are not marked in the cytoplasm, nor does the cytoplasm contain more than a few secretory inclusions. Large numbers of nucleoli are not seen in transit to the cytoplasm and, in particular, the inner region of the gland is seen to be breaking down in the way formerly held to characterise healthy holocrine secretion. The differences between active and degenerating silk gland cells are thus quite clear, and it seems better to ascribe a function in silk production to the migrated nucleoli rather than to use their presence in the cytoplasm to argue for some unspecified adjustment behaviour by the nuclei of the actively silk producing cells.

VI. Summary

The mature larva of *Dianous coerulescens* Gyllenhal spins a cocoon within which it pupates. The cocoon is a double structure and the silk used in its construction comes from paired glands which open on to the ninth tergite near the bases of the anal cerci. The glands are syncytial in nature, each containing several hundred nuclei. In young larvae these nuclei are ovoid with an easily resolved nuclear membrane. When silk is being secreted the nuclei are larger and amoeboid, with an increased nucleolar content. Silk appears to be produced, at least in part, by solution in the gland cytoplasm of nucleoli which have migrated from these amoeboid nuclei.

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